The following document consists of supplemental information supporting the findings communicated in:

CIS-REGULATORY CONTROL OF THE INITIAL NEUROGENIC PATTERN OF *ONECUT* GENE EXPRESSION IN THE SEA URCHIN EMBRYO

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3 online resources related to this study

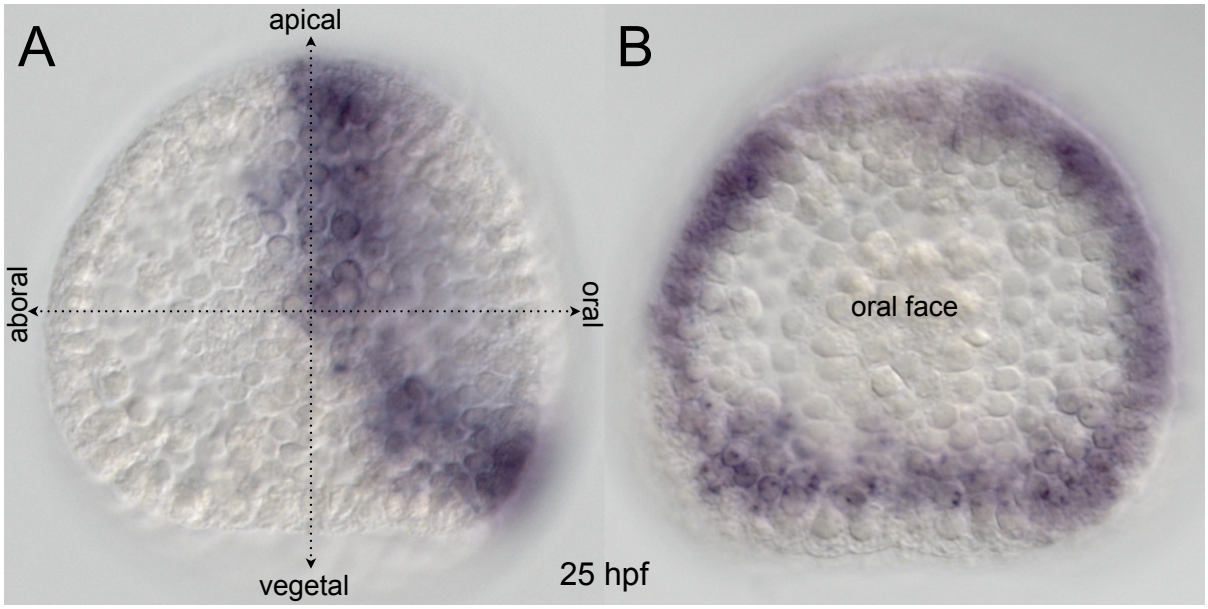
Transcriptional dynamics

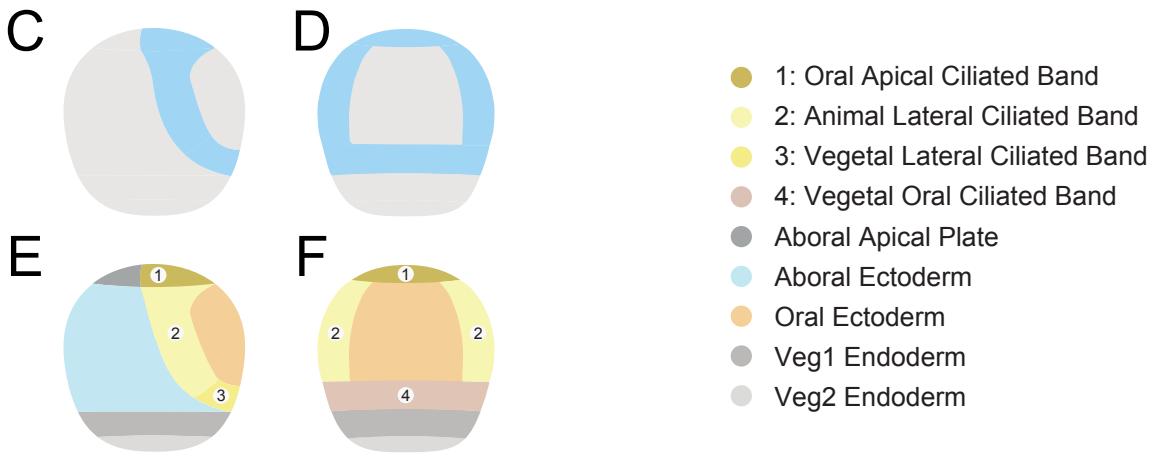
1. http://www.spbase.org:3838/quantdev/
2. http://echinobase.org/hd-tc/plot.cgi

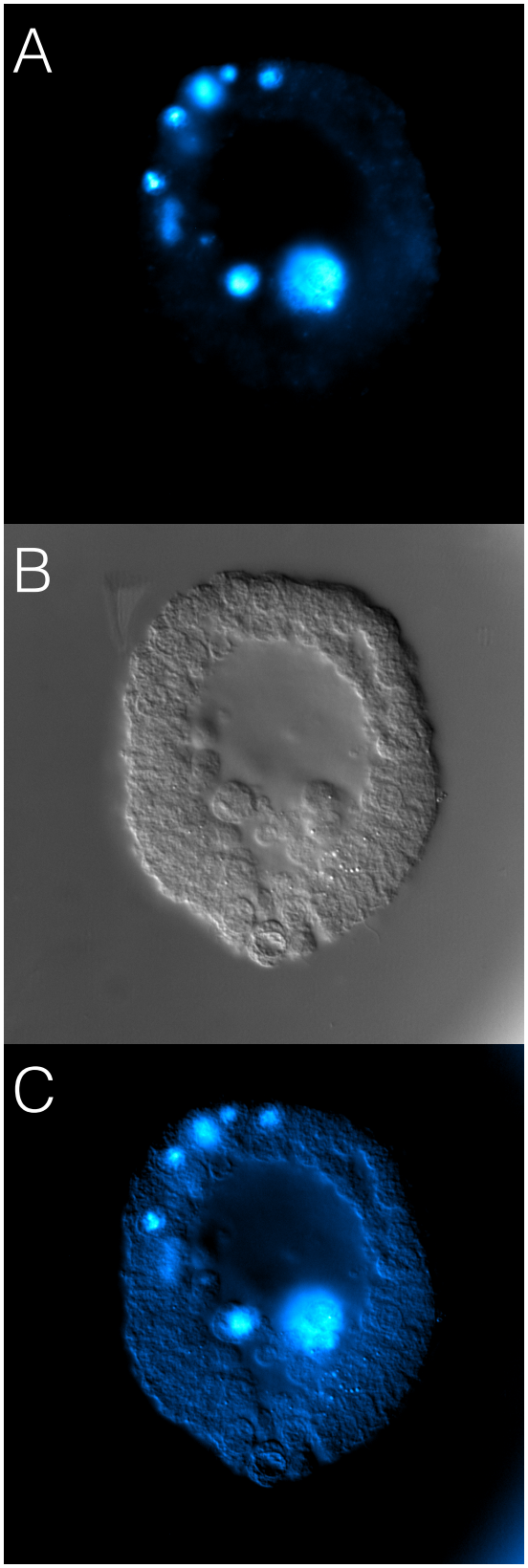
Cell-type specific gene cohorts

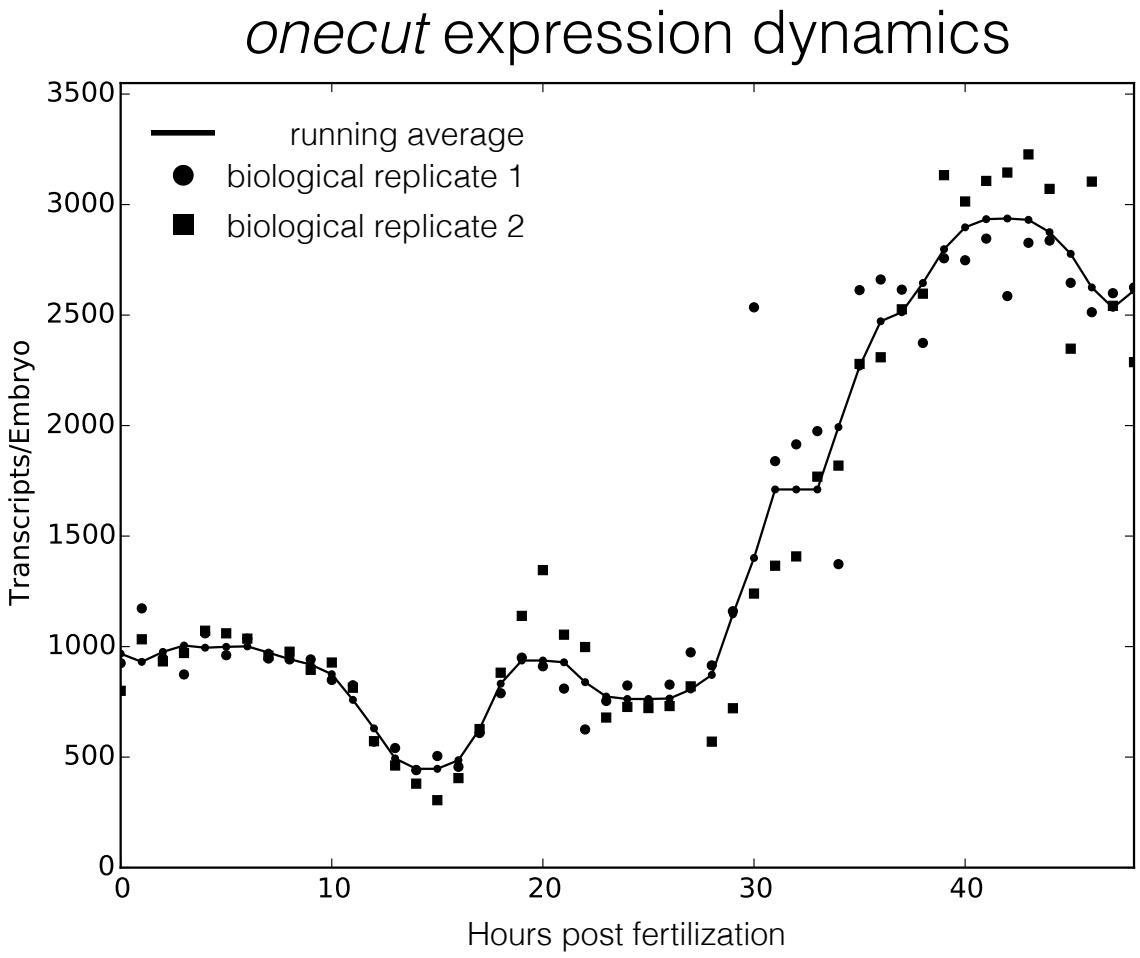
3. http://www.echinobase.org/SpBase/rnaseq/embryonic_territory.html

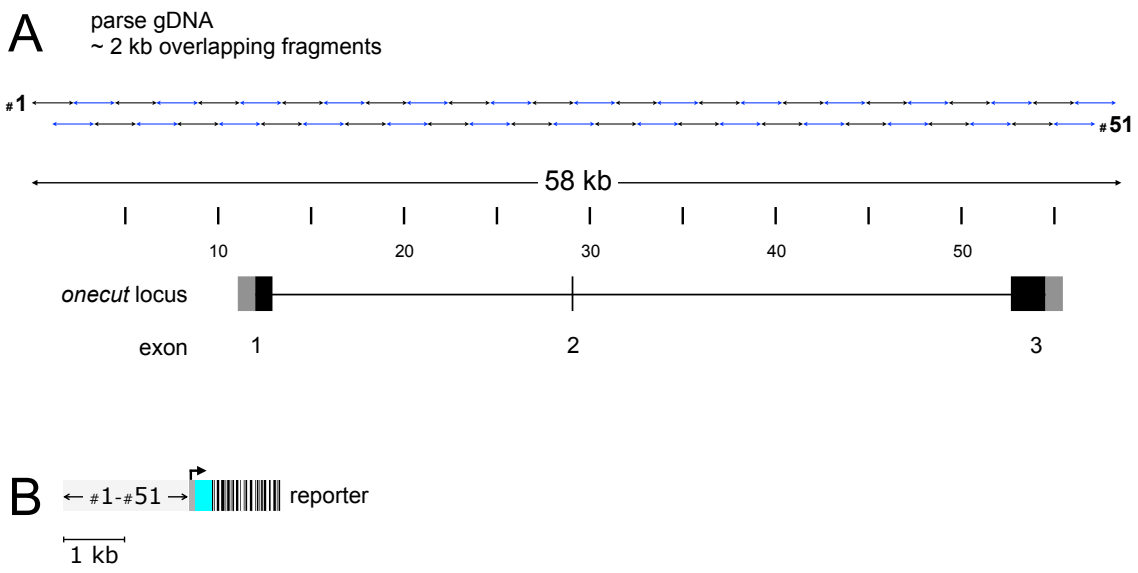
INDEX


A
apical
aboral
oral
vegetal
B
oral face
25 hpf


C
D
E
F
1
2
3
4
1: Oral Apical Ciliated Band
2: Animal Lateral Ciliated Band
3: Vegetal Lateral Ciliated Band
4: Vegetal Oral Ciliated Band
Aboral Apical Plate
Aboral Ectoderm
Oral Ectoderm
Veg1 Endoderm
Veg2 Endoderm


A
B
C


onecut expression dynamics
running average
biological replicate 1
biological replicate 2
Transcripts/Embryo
Hours post fertilization
0
500
1000
1500
2000
2500
3000
3500
0
10
20
30
40


A
parse gDNA
~ 2 kb overlapping fragments
#1
#51
58 kb
10
20
30
40
50
onecut locus
exon
1
2
3
B
←#1-#51→
reporter
1 kb

Proximal *cis*-regulatory module 384 bp

GTTTAACTGTGCTCAGTTATGTAAACATATCGAATTTCTCCCCACAGTCTGTCTAATTTATCGAACAAACATTTTCTAATCTCCCGCCCGAAACTAGGAC
CAAATTGACACGAGTCAATACATTTGTATAGCTTAAAGAGGGGTGTCAGACAGATTAAATAGCTTGTTTGTAAAAGATTAGAGGGCGGGCTTTGATCCTG

ATTCGTGCTAAGCTCCAAAAGACGTTGACCTGTGTAGGTTAACGCGTAGTCTGTTTAAAACTAAAGCATATGGCAGGTTAACAATACATGGCAATATACC
TAAGCACGATTCGAGGTTTTCTGCAACTGGACACATCCAATTGCGCATCAGACAAATTTTGATTTCGTATACCGTCCAATTGTTATGTACCGTTATATGG

CCCTGTTCTGCACACACAGAATTGTTACAATGAAGTTGTTACTTTGAACAGGTAGTTAGTGAGACAATTGCGCATGCGTTGTGGAACGTAACAAAGGCTT
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TCATACATGGTTGAGCGAGCTGAGTCCTGTAGGGGTTTTACGTGTAATGCTATTGTAATGTCATTGAGGGGAGAGAGAGAAAGA
AGTATGTACCAACTCGCTCGACTCAGGACATCCCCAAAATGCACATTACGATAACATTACAGTAACTCCCCTCTCTCTCTTTCT

Intron C (central) *cis*-regulatory module 1747 bp

GGCTATTTGGCAATCTTAAGAAGATGAAGACAGGCCTAATGATTATGGGATGAGTATCGCTAGCAACCGAGCTAAGCTCCAGAAATGTGATGGACCTCGGTCCTCCACGTCTATTACTCT
CCGATAAACCGTTAGAATTCTTCTACTTCTGTCCGGATTACTAATACCCTACTCATAGCGATCGTTGGCTCGATTCGAGGTCTTTACACTACCTGGAGCCAGGAGGTGCAGATAATGAGA

TCTGGTCTCCCTTAAAGGGATATATCAACTGTATTTATGAAAGATGTAGTTTATTCATGCCATAATCAATTGGAATACAGTGTACAACCTGAGAAATGAACAGAGGCATGCAAATCAGTA
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TCTTTTTGCCACCAGAAATATCTGTCCACCAAAAAGATAAGTCCACCAACGAGTTTGTCCAAACTGACACAAGGGAAAGTTTTCGTACAAAAGAGAGTAAGTTACAAAAGAAAAGGGTAG

ATTATAGGCTCATCAATTTACTTTTGCATGGCATCATCAATCGAACATACACAGTGACTTTATGTTTTTCTAGAATATTTCCTTGTTAAAAAAGCTCAA
TAATATCCGAGTAGTTAAATGAAAACGTACCGTAGTAGTTAGCTTGTATGTGTCACTGAAATACAAAAAGATCTTATAAAGGAACAATTTTTTCGAGTT

intervening sequence was left intact so as to not disrupt Exon 2

GGGCTACGGCATGGGGAGATGGATAGATCGAGAGGGAAAATTGATTATATCATTGTTATATCCATTTTAACAGGCGATAAATTATTGATATACACATCCCACAGTGATTG
CCCGATGCCGTACCCCTCTACCTATCTAGCTCTCCCTTTTAACTAATATAGTAACAATATAGGTAAAATTGTCCGCTATTTAATAACTATATGTGTAGGGTGTCACTAAC

ATTAATTATTCATAATCAATAAATGTTTAATTGACCCTTTTCAATTAACAAAACAATCAAAATATCAATCATTTAAGGTTGTCTTCCCCGATGTTTCCGTTTACACGGCC
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ACCAATTACTAAATATTGAGCAAAATGAAGCCTTGTCATAATTATGAGCTATTACACGACATGCAATCATGCATGCTAAACGATATCCTCCTTTACAAGACTCTATTCAA
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AATGATCTTTTTTAGGACTTGAGCTCACAATAGCCTTTTTGTAAGTGAGGATTTAAAATTTAATGCAAGAAACAAAGAACAACGCCAGCAGTAATTGAAATTGTTAGCTG
TTACTAGAAAAAATCCTGAACTCGAGTGTTATCGGAAAAACATTCACTCCTAAATTTTAAATTACGTTCTTTGTTTCTTGTTGCGGTCGTCATTAACTTTAACAATCGAC

ATAGACAATGTAACATCACTGATGCGTTTTGATAGTTTGGTAGGGGAGGTAGAAAATGCCTGTATGAAAATTCTATTTCGAGCTGCAAGGATAGCAGTCATTTAATGATA
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TTTGACAATTTTTTAAACAGTATCTGAATTCTGGAAATATGCTGAAATACATCACATTTTTACAAACCTGCAGAGGACAATACTTTACTCTTACAAAATGATATCATTTT
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AATCATCAATTTTAATTAATATAACTATAAGTAAATTATTTGAGATGATATTTCCTTTCAAAATATTGGCATACATCTCTTAACACATTTCTTAAGCGTGAATAGGAATT
TTAGTAGTTAAAATTAATTATATTGATATTCATTTAATAAACTCTACTATAAAGGAAAGTTTTATAACCGTATGTAGAGAATTGTGTAAAGAATTCGCACTTATCCTTAA

TCAAAGGGTTTTTAGCTATTTCTCATCTCAGTAAATATATCCATTCCTATTAATGCGGCCTTGGCAGAAATACACATGTATGATGGCAATTTCAGTTTATATGCGAAATG
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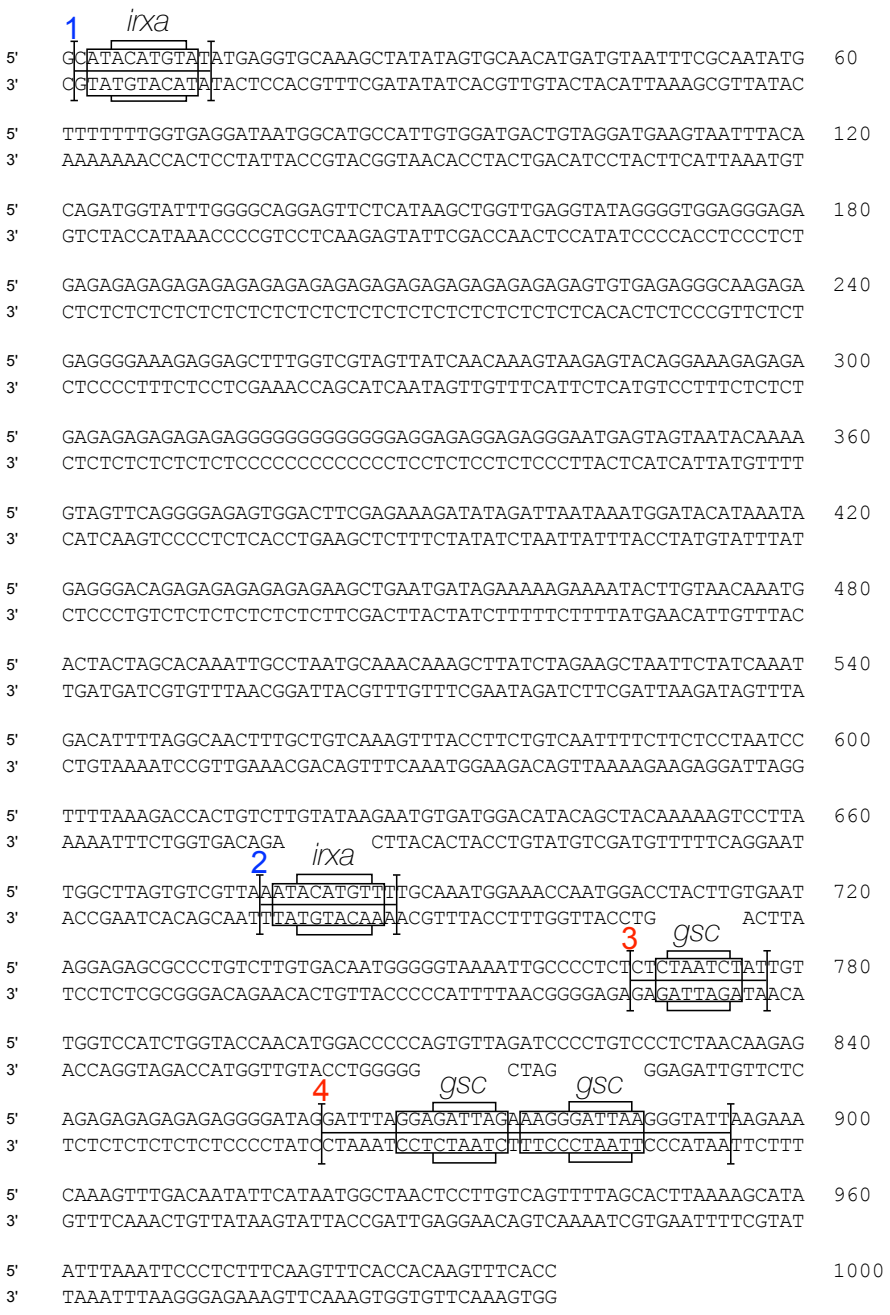
GTGAAATTTTGCTATTGTGACTACAATTTTTCCTTTGCTATTTTTCTATTGAAGTGACCATCATGATGACTGAAATAA
CACTTTAAAACGATAACACTGATGTTAAAAAGGAAACGATAAAAAGATAACTTCACTGGTAGTACTACTGACTTTATT

Intron D (distal) *cis*-regulatory module 1000 bp

A

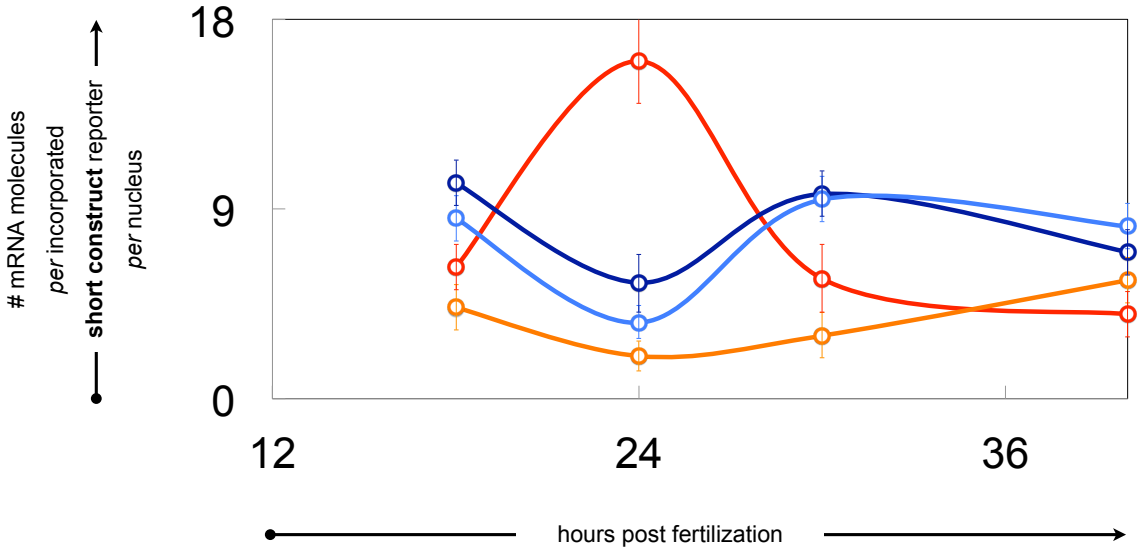


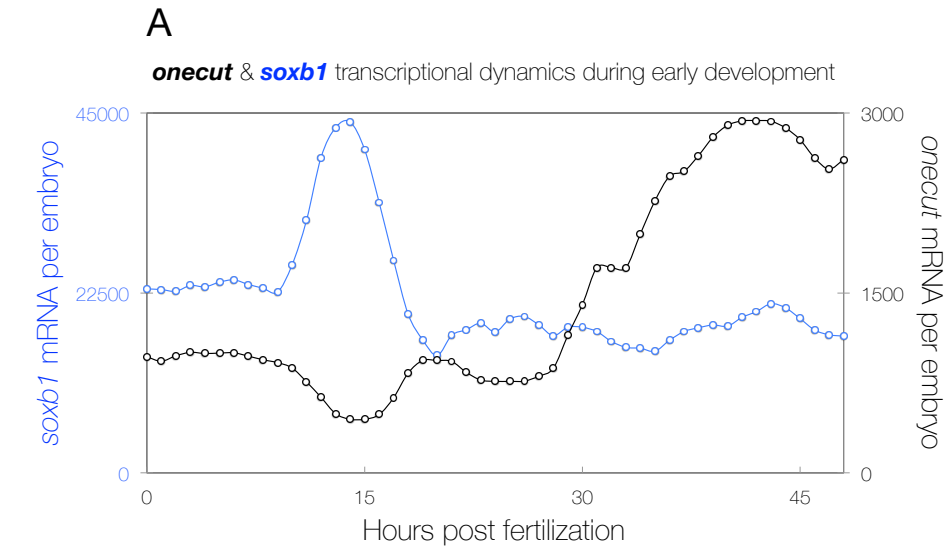
B

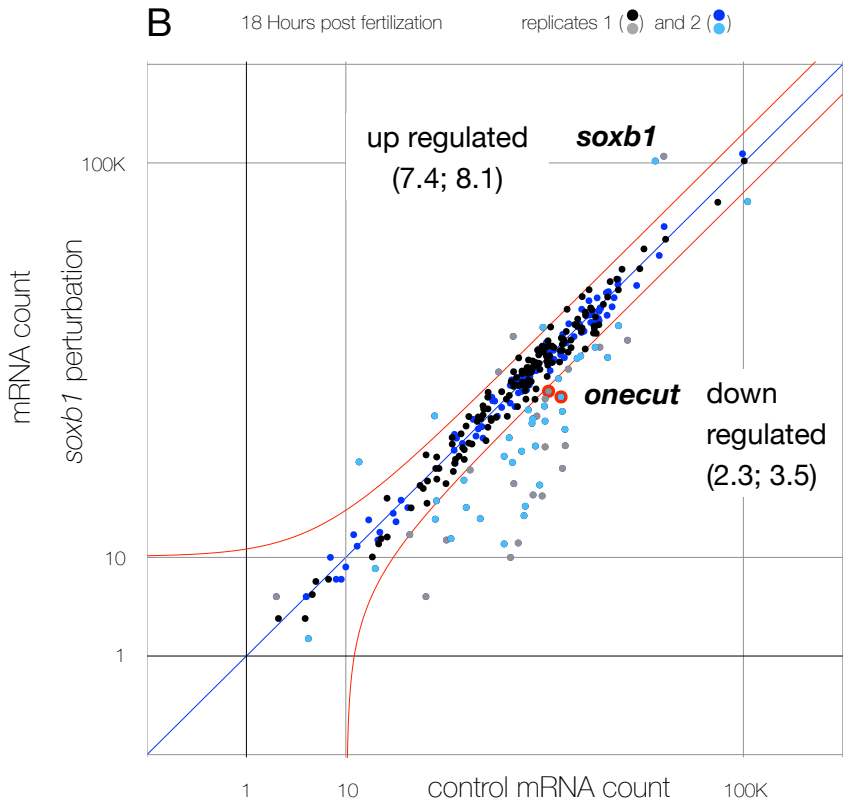
TGCCACGCTAA
ACGGTGCGATT
C H A N

C

TGATGATGCCACGCTAATGGAGAGGACTGATGA
ACTACTACGGTGCGATTACCTCTCCTGACTACT
. . C H A N G E D . .


mRNA molecules
per incorporated
short construct reporter
per nucleus
18
9
0
12
24
36
hours post fertilization


A
onecut & soxb1 transcriptional dynamics during early development
45000
22500
0
soxb1 mRNA per embryo
3000
1500
0
onecut mRNA per embryo
0
15
30
45
Hours post fertilization


B
18 Hours post fertilization
replicates 1 ( ) and 2 ( )
up regulated
(7.4; 8.1)
soxb1
onecut
down
regulated
(2.3; 3.5)
100K
10
1
mRNA count
soxb1 perturbation
1
10
control mRNA count
100K

DNA Reporter Construct	qPCR Primer Pair (Nam et al 2010)	Embryos per Measurement	Timepoint (Hpf)	Replicate	cDNA Ct	gDNA Ct	mRNA per construct
Negative control (N3P)	Tag05	6	18	1	29.4	21.6	2.3
Negative control (N3P)	Tag05	6	18	2	29.1	21.6	
Negative control (N3P)	Tag05	6	18	3	29.2	21.6	
Negative control (N3P)	Tag05	6	24	1	29.2	20.8	2.8
Negative control (N3P)	Tag05	6	24	2	29.9	20.8	
Negative control (N3P)	Tag05	6	24	3	29.7	20.9	
Negative control (N3P)	Tag05	6	30	1	27.0	20.8	5.2
Negative control (N3P)	Tag05	6	30	2	28.1	20.8	
Negative control (N3P)	Tag05	6	30	3	28.6	20.8	
Negative control (N3P)	Tag05	6	35	1	29.1	20.4	2.1
Negative control (N3P)	Tag05	6	35	2	28.9	20.4	
Negative control (N3P)	Tag05	6	35	3	29.5	20.6	
#5 - Tag02	Tag02	6	18	1	27.5	21.4	19.3
#5 - Tag02	Tag02	6	18	2	27.5	21.3	
#5 - Tag02	Tag02	6	18	3	28.2	28.6	
#5 - Tag02	Tag02	6	24	1	27.7	20.5	10.4
#5 - Tag02	Tag02	6	24	2	27.3	20.6	
#5 - Tag02	Tag02	6	24	3	32.1	26.1	
#5 - Tag02	Tag02	6	30	1	26.5	20.5	42.6
#5 - Tag02	Tag02	6	30	2	26.3	20.6	
#5 - Tag02	Tag02	6	30	3	29.6	30.9	
#5 - Tag02	Tag02	6	35	1	26.0	19.9	59.6
#5 - Tag02	Tag02	6	35	2	26.1	19.9	
#5 - Tag02	Tag02	6	35	3	25.9	27.6	
Negative control (N3P)	Tag01	6	18	1	26.2	18.8	4.1
Negative control (N3P)	Tag01	6	18	2	25.8	18.9	
Negative control (N3P)	Tag01	6	18	3	26.0	18.7	
Negative control (N3P)	Tag01	6	30	1	26.2	17.9	2.3
Negative control (N3P)	Tag01	6	30	2	25.8	18.0	
Negative control (N3P)	Tag01	6	30	3	25.9	18.0	
Negative control (N3P)	Tag01	6	38	1	26.0	19.3	3.4
Negative control (N3P)	Tag01	6	38	2	26.8	18.8	
Negative control (N3P)	Tag01	6	38	3	26.4	18.8	
#14 - Tag03	Tag03	6	18	1	25.0	19.2	13.8
#14 - Tag03	Tag03	6	18	2	25.0	19.2	
#14 - Tag03	Tag03	6	18	3	25.1	20.6	
#14 - Tag03	Tag03	6	30	1	25.3	18.5	4.7
#14 - Tag03	Tag03	6	30	2	25.5	18.5	
#14 - Tag03	Tag03	6	30	3	25.5	18.5	
#14 - Tag03	Tag03	6	38	1	25.0	19.3	10.0
#14 - Tag03	Tag03	6	38	2	25.6	19.4	
#14 - Tag03	Tag03	6	38	3	24.9	19.4	
Negative control (N3P)	Tag05	6	18	1	28.7	20.8	4.0
Negative control (N3P)	Tag05	6	18	2	29.1	20.8	
Negative control (N3P)	Tag05	6	18	3	29.1	20.9	
Negative control (N3P)	Tag05	6	24	1	27.2	18.5	2.6
Negative control (N3P)	Tag05	6	24	2	27.3	18.4	
Negative control (N3P)	Tag05	6	24	3	26.9	18.4	
Negative control (N3P)	Tag05	6	33	1	26.5	18.3	3.1
Negative control (N3P)	Tag05	6	33	2	26.8	18.3	
Negative control (N3P)	Tag05	6	33	3	26.9	18.3	
Negative control (N3P)	Tag05	6	40	1	27.3	17.6	1.9
Negative control (N3P)	Tag05	6	40	2	27.2	17.6	
Negative control (N3P)	Tag05	6	40	3	26.8	17.6	
#23 - Tag13	Tag13	6	18	1	26.4	20.4	16.6
#23 - Tag13	Tag13	6	18	2	26.4	20.5	
#23 - Tag13	Tag13	6	18	3	27.6	26.0	
#23 - Tag13	Tag13	6	24	1	24.4	17.9	11.3
#23 - Tag13	Tag13	6	24	2	24.4	17.8	
#23 - Tag13	Tag13	6	24	3	25.3	30.7	
#23 - Tag13	Tag13	6	33	1	23.8	17.9	21.1
#23 - Tag13	Tag13	6	33	2	23.6	18.5	
#23 - Tag13	Tag13	6	33	3	25.6	Undetermined	
#23 - Tag13	Tag13	6	40	1	24.5	17.3	11.5
#23 - Tag13	Tag13	6	40	2	24.6	17.9	
#23 - Tag13	Tag13	6	40	3	29.3	27.8	

	DNA Sequence Assessed	qPCR Primer Pair	Embryos per Measurement	Timepoint (Hpf)	average mRNA per construct
Embryos consecutively harvested in time & space	onecut BAC CRE knock out Prox+intronCa+intronCb+intronD	GFP	6	18	**11.8**
			6	24	3.9
			6	30	3.8
			6	35	3.3
	onecut BAC WT internal control	mCherry	6	18	**31.1**
			6	24	**26.7**
			6	30	**54.4**
			6	35	**58.0**
Embryos consecutively harvested in time & space	onecut BAC CRE knock out Prox	GFP	6	18	1.6
			6	24	0.7
			6	30	1.3
			6	35	1.1
	onecut BAC WT internal control	mCherry	6	18	**11.2**
			6	24	5.4
			6	30	**10.1**
			6	35	**24.8**
Embryos consecutively harvested in time & space	onecut BAC CRE knock out intronCa+intronCb	GFP	6	18	**21.4**
			6	24	0.0
			6	30	**11.0**
			6	35	**15.7**
	onecut BAC WT internal control	mCherry	6	18	**17.2**
			6	24	6.9
			6	30	**49.6**
			6	35	**28.1**
Embryos consecutively harvested in time & space	onecut BAC CRE knock out intronD	GFP	6	18	**36.6**
			6	24	**136.7**
			6	30	**44.0**
			6	35	**28.0**
	onecut BAC WT internal control	mCherry	6	18	**24.5**
			6	24	6.9
			6	30	**26.3**
			6	35	**45.1**

Hours post fertilization	Ubiquitin abundance used for each timepoint [mRNA per embryo]
0	63447
1	61663
2	63864
3	66251
4	64934
5	64934
6	64694
7	62433
8	62433
9	63940
10	68225
11	74965
12	81954
13	87431
14	91359
15	93475
16	94906
17	94906
18	93943
19	86843
20	76826
21	63244
22	54940
23	49600
24	48050
25	47137
26	47735
27	46240
28	45815
29	45503
30	45191
31	44002
32	42474
33	43498
34	42170
35	40607
36	40379
37	41503
38	41410
39	41564
40	41842
41	42251
42	42151
43	41069
44	42420
45	41779
46	39230
47	37374
48	39424

Supplemental Figure Legends

Supplemental Figure 1. **Pregastrular *onecut* gene expression pattern**. (A-B) RNA *in situ* hybridization reveals spatial localization of *onecut* mRNA at mesenchyme blastula stage. (A) Expression pattern of *onecut* at 25 hpf observed from lateral perspective. Spatial coordinates illustrate the separation of oral from aboral ectoderm by the *onecut* expression domain, and exclusion of *onecut* expression from the vegetal endodermal region of the embryo. The embryonic territory from which the ciliated band will arise is first delineated by zygotic *onecut* expression at this time. (B) Same embryo as in A observed from an oral perspective. The oral ectoderm, referred to as the oral face (labeled), lies within the *onecut* expression boundary. Zygotic transcription of *onecut* is first visible by RNA *in situ* hybridization at 24 hpf; prior to this maternally deposited *onecut* mRNA is ubiquitously distributed, obscuring the zygotic expression pattern. (C-F) Schematic representation of the ciliated band, its subdomains, and adjacent embryonic territories. (C, D) Diagram illustrating the position of the ciliated band (blue) with respect to the embryo, as viewed from a lateral and oral perspective as in A and B, respectively. (E, F) Ciliated band subdomains relative to adjacent embryonic territories (color-coded). These diagrams are simplified by omitting territorial subdivisions within the ectoderm (Li et al., 2014). Note: Images shown have been amended from Barsi *et al*. 2015.

Supplemental Figure 2. **Pregastrular expression of *onecut*:gfp BAC reporter**. (A) Fluorescent GFP expression (pseudo-colored blue) observed across half of the ciliated band domain. (B) DIC image of the transgenic embryo (20 hpf) expressing the GFP signal shown in A. (C) Composite image generated from merging A and B reveals GFP expression relative to the pregastrular embryo. Note: GFP does not recapitulate the full extent of the ciliated band due to mosaic transgene incorporation inherent to this model organism.

Supplemental Figure 3. ***Onecut* expression dynamics**. mRNA abundance profiles throughout early embryogenesis as measured by the nCounter Analysis System. Circular and square data points each represent a biological replicate. Solid curve reflects the running average between replicates. Data amended from (Materna *et al*., 2010).

Supplemental Figure 4. ***Cis*-regulatory analysis of the *onecut* gene**. (A) Identification: 58 kb of genomic sequence including the *onecut* locus was parsed into overlapping fragments averaging 2.23 kb in length (#1-#51), each of which was indexed according to a *bar-coded* expression vector in the form of a unique DNA sequence tag (Nam et al., 2010) and assessed for its capability to drive reporter expression *in vivo*. (B) Structure of the reporter construct: light gray, genomic DNA fragment; dark gray, *onecut* basal promoter including the 5′ UTR; turquoise, GFP CDS; bar-code, DNA sequence tag.

Supplemental Figure 5. **Proximal *cis*-regulatory module**. DNA sequence 384 bp in length corresponding to *onecut*'s proximal *cis*-regulatory module.

Supplemental Figure 6. **Intron C *cis*-regulatory module**. DNA sequence 1747 bp in length corresponding to *onecut*'s intron C (central) *cis*-regulatory module. Note: The intervening sequence (not shown) was left intact during the deletion of intron C *cis*-regulatory module (as shown in Figure 3 B) so as to preserve Exon 2 and its associated splice acceptor/donor sites.

Supplemental Figure 7. **Intron D *cis*-regulatory module**. (A) DNA sequence 1000 bp in length corresponding to *onecut*'s intron D (distal) *cis*-regulatory module. Repressor target sites enumerated 1-4. *lrxa* target sites are indicated in blue numerals (1, 2) and *Gsc* target sites in red numerals (3, 4). DNA within the windows enumerated 1-3 were permutated to the sequence shown in (B). DNA within window 4 was permutated to the sequence shown in (C).

Supplemental Figure 8. **Expression dynamics of Intron D *cis*-regulatory module reporter construct**. qPCR measurements depicting *gfp* expression in transgenic embryos. Baby blue curve, initial DNA fragment 2 kb in length harboring the intron D *cis*-regulatory module; Navy blue curve, intron D *cis*-regulatory module 1 kb in length; Red curve, mutant version of intron D *cis*-regulatory module with compromised *Gsc* TFBS (*transcription factor binding sites*) (1 kb); Orange curve, negative control DNA fragment (894 bp).

Supplemental Figure 9. **Ancillary evidence for regulatory relation between *soxb1* and *onecut***. (A) mRNA abundance profiles throughout early embryogenesis. Black curve shows endogenous *onecut* expression; data points refer to mRNA molecules *per* embryo shown on right ordinate. Blue curve reflects endogenous *soxb1* expression; left ordinate. Data redrawn from published measurements (Materna et al., 2010). Notice that total *per* embryo *soxb1* levels remain extremely high throughout. (B) Down regulation of multiple regulatory genes in embryos bearing *soxb1* morpholino, measured simultaneously by the nCounter Analysis System. Transcript levels for 181 regulatory genes and signaling ligands are compared between control embryos (abscissa) and *soxb1* morpholino treated embryos (ordinate). Each sample contained RNA extracted from 33 embryos. Red envelope indicates the maximum range of scatter observed in large numbers of control runs with this instrument, amounting to maximum two-fold variation in the linear range of the data shown. Genes that fall outside these limits, represented by light blue and gray dots, are significantly affected by the morpholino treatment. The vast majority of the genes assessed are unaffected, dark blue and black dots. Light blue and gray vs. dark blue and black represent two genetically distinct replicates.

Supplemental Table 1. **CRM sequence-tag expression values**. Raw data from the initial screen pertaining to the *cis*-regulatory analysis depicted in Supplemental Figure 4.

Supplemental Table 2. **BAC CRM deletion construct expression values**. Absolute expression values computed from qPCR measurements as previously reported in Revilla-i-Domingo *et al*. 2004. Different BAC CRM deletion constructs harboring the GFP CDS were co-injected with the wild type BAC containing an alternate fluorophore (mCherry). Note: *Sp*. embryos display biological variance in terms of DNA incorporation, developmental timing and the degree to which they express transgenes. Because of this, experimental results will vary from one batch of embryos to the next and the use of an internal control is necessary for correct interpretation.

Supplemental Table 3. **Ubiquitin expression dynamics**. Average values from published measurements (Materna et al., 2010) used as a standard for the computation of absolute reporter expression.

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